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Comparison of cigarette smoke-induced lipid peroxidation *in vitro* and *in vivo**

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With 6 figures

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Introduction

Results from *in vitro* studies (Frei et al. 1991; Niki et al. 1993) suggest that cigarette smoke can cause or promote lipid peroxidation. Lipid peroxidation yields lipid hydroperoxides as major initial reaction products. Lipid peroxidation and protection mechanisms in biological membranes are schematically presented in fig. 1.

The combination of an HPLC system with an ultrasensitive analytical system based on the detection of chemiluminescence emitted by isoluminol in the presence of microperoxidase and lipid hydroperoxides is well known for its picomole-level sensitivity (Yamamoto et al. 1987). Using this method, which we adapted to detect phospholipid hydroperoxides (PLOOH), we compared the cigarette smoke-induced increase of PLOOH in mouse embryo BALB/c 3T3 cells *in vitro* and in rat lungs *in vivo*.

Material and methods

In vitro: 36 puffs of mainstream whole smoke, generated from the standard reference cigarette 2R1, were bubbled through 18 ml PBS. For the generation of the gas vapor/phase-bubbled PBS, a Cambrige filter was placed in the smoke stream. Both solutions were diluted with PBS to obtain the required concentrations. Mouse embryo BALB/c 3T3 cells (~ 1 x 10^7 cells) were incubated with the respective smoke solutions or standard oxidants for 30 min at 37 °C. After centrifugation, supernatants were used for lactate dehydrogenase (LDH) determination, and phospholipids were extracted from the cell pellets with chloroform/methanol in the presence of 1 mg probucol. In order to exclude artifacts during the phospholipid isolation procedure, lysophosphatidylcholine hydroperoxide, prepared from soybean lysophosphatidylcholine, was used as an internal standard (IS).

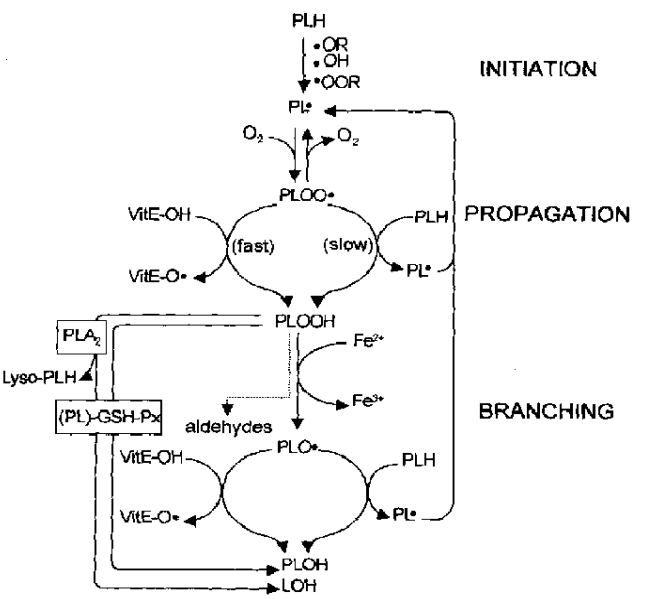

Fig. 1. Lipid peroxidation and protection mechanisms in biological membranes; PLH = phospholipid; LOH = fatty acid hydroxide; VitE-OH = vitamine E; (PL)-GSH-Px = (phospholipid hydroperoxide) glutathione peroxidase; PLA_2 = phospholipase A_2.

* Poster, presented at the 5th International Inhalation Symposium, Hannover, Germany, 20–24 February 1995.

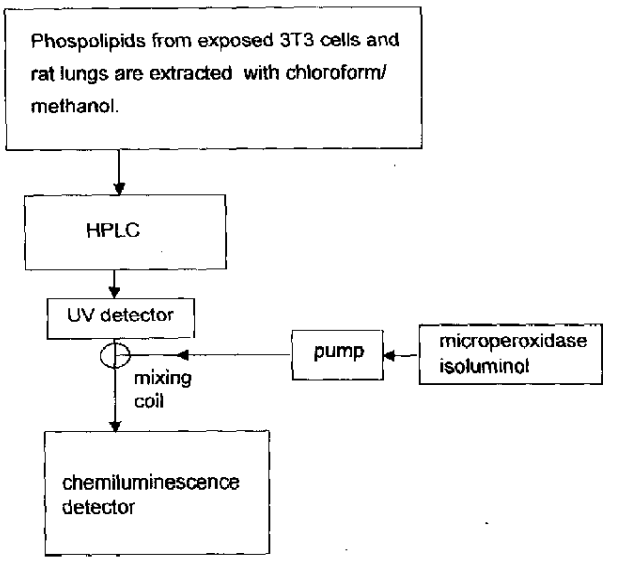

Fig. 2. Schematic diagram of the determination of phospholipid hydroperoxides by postcolumn chemiluminescence detection.

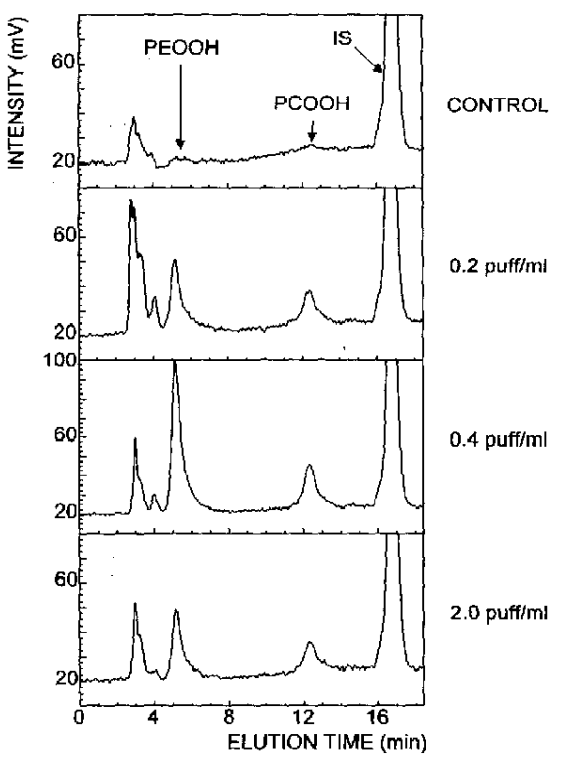

Fig. 3. Phosphatidylethanolamine hydroperoxide (PEOOH) and phosphatidylcholine hydroperoxide (PCOOH) formation in 3T3 cells exposed to gas/vapor phase-bubbled PBS for 30 min at 37 °C; HPLC separation of phospholipid classes in combination with selective chemiluminescence detection of phospholipid hydroperoxides.

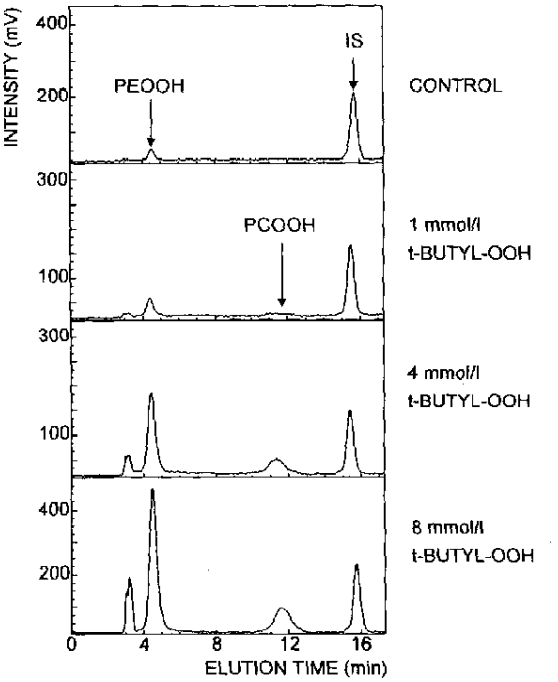

Fig. 4. Phosphatidylethanolamine hydroperoxide (PEOOH) and phosphatidylcholine hydroperoxide (PCOOH) formation in 3T3 cells exposed to t-butylhydroperoxide for 30 min at 37 °C; HPLC separation of phospholipid classes in combination with selective chemiluminescence detection of phospholipid hydroperoxides.

In vivo: 5-week-old, male Sprague Dawley rats were head-only exposed to 40 µg TPM/l of mainstream smoke (MS) of the standard reference cigarette 1R4F for 63 days, 6 hours per day, 7 days per week. Rats were sacrified immediately after final exposure, and the right lungs were removed and stored at −70 °C. For the determination of PLOOH, lungs were homogenized, and lipids were extracted with chloroform/methanol in the presence of 1 mg probucol.

PLOOH analysis: The different phospholipid classes were separated by HPLC on Kromasil Si, 5 µm, using a water-methanol-acetonitrile solvent system in combination with a chemiluminescence detector for the selective determination of PLOOH. Fig. 2 shows a schematic diagram of the HPLC-isoluminol chemiluminescence assay.

Results and discussion

In vitro: Whole smoke-bubbled PBS did not induce lipid peroxidation up to a concentration of 2 puff/ml. However, lipid peroxidation was induced by gas/vapor-phase-bubbled PBS. The data indicate a threshold level at 0.1 puffs/ml, a maximum effect at 0.4 puffs/ml and a decrease in lipid peroxidation at higher puff concentrations (fig. 3, fig. 5B). In contrast, exposure to t-butylhydroperoxide, a

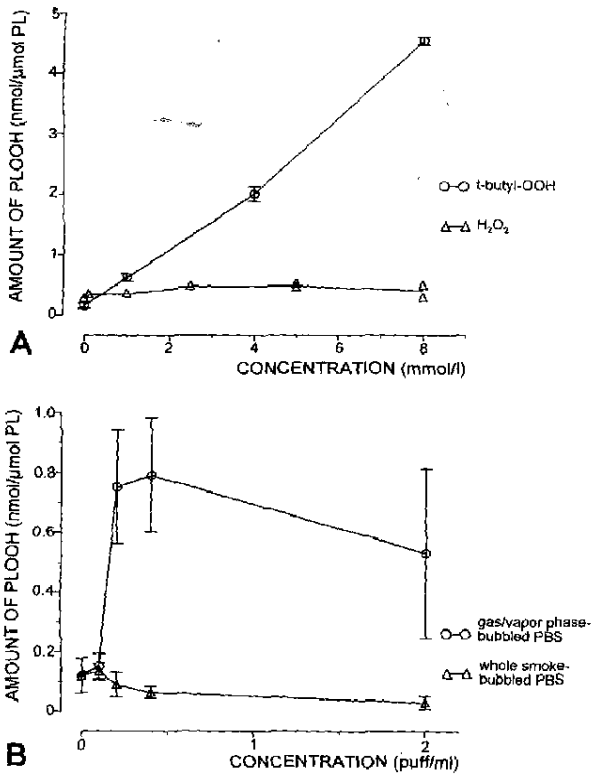

Fig. 5. PLOOH (sum of phosphatidylethanolamine hydroperoxide and phosphatidylcholine hydroperoxide) formation in 3T3 cells; (A) Effect of t-butylhydroperoxide (mean ± SE, n = 3) and H_2O_2; (B) Effect of gas/vapor phase-bubbled PBS and whole smoke-bubbled PBS (mean ± SE, n = 3).

standard oxidant, dose dependently increased the amount of oxidated phospholipids (fig. 4, fig. 5A). No lipid peroxidation was detected after cell exposure to hydrogen peroxide up to a concentration of 8 mmol/l (fig. 5A). Following exposure, no indications of cell damage, measured by LDH activity in the supernatants, were detected. A possible explanation for the inability of whole smoke-bubbled PBS to induce the formation of phospholipid hydroperoxides *in vitro* is the covering of oxidative effects of smoke radicals by phenolic antioxidants, components of the particulate fraction of cigarette smoke.

In vivo: Mainstream whole smoke at a dose of 40 µg TPM/l approximately doubled the amount of phospho-

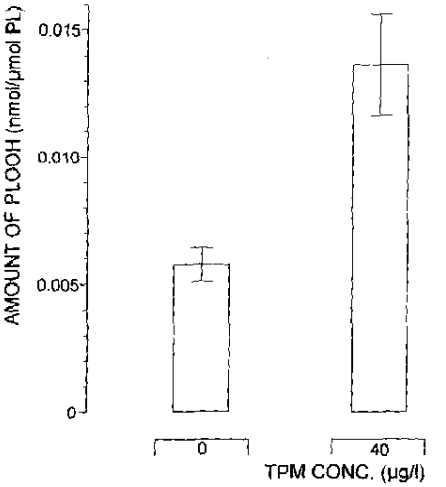

Fig. 6. Amount of phospholipid hydroperoxides in rat lungs after MS inhalation (mean ± SE, n = 8).

lipid hydroperoxides in rat lungs compared to the spontaneous level in sham-exposed rats (fig. 6). This could mean that in the rat lungs, particles were separated from gas phase components. The persistence of smoke-induced oxidative effects in rat lungs remains to be investigated.

The comparison of *in vitro*- with *in vivo*-induced lipid peroxidation indicates that gas/vapor phase-bubbled PBS is a better model than whole smoke-bubbled PBS for *in vivo* whole smoke-induced lipid peroxidation in rat lungs.

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